Novel Mutations in *ALOX12B* in Patients with Autosomal Recessive Congenital Ichthyosis and Evidence for Genetic Heterogeneity on Chromosome 17p13

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We report clinical and molecular findings in 20 patients from 11 families with autosomal recessive congenital ichthyosis (ARCI) linked to chromosome 17p13, and attributed to mutations in the *ALOX* gene cluster, which includes three lipoxygenase genes, *ALOXE3*, *ALOX12B*, and *ALOX15B*. We identified six novel missense mutations and one novel deletion leading to a premature stop codon in *ALOX12B* in only six out of the 11 families which led us to investigate a possible implication of *ALOX15B*. Mutation analysis of this gene, as well as *ALOXE3*, which is known to be mutated in some cases of ARCI, failed to reveal causative mutations in the five remaining ARCI families, indicating that other genes on chromosome 17p13 may be involved in this disease. However, by adding new variants to the repertoire of *ALOX12B* mutations in non-bullous congenital ichthyosiform erythroderma, our data contribute to an enlargement of the spectrum of mutations for the development of efficient molecular genetic tests for analysis of at risk individuals whose carrier status is unknown.

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INTRODUCTION

Autosomal recessive congenital ichthyosis (ARCI) is a clinically and genetically heterogeneous group of disorders of keratinization characterized by skin desquamation over the whole body, often associated with erythema (Williams and Elias, 1985; Traupe, 1989). It is a severe condition with an estimated prevalence of one in 300,000 newborns and most of the patients are born as collodion babies. To date, six genes for ARCI have been identified, *TGM1* (MIM 242300) on chromosome 14q11 (Huber *et al.*, 1995; Russell *et al.*, 1995), *ABCA12* (MIM 601277) on chromosome 2q34–q35 (Lefèvre *et al.*, 2003), *ichthyin* on chromosome 5q33 (Lefèvre *et al.*, 2004), *ALOXE3* and *ALOX12B* (MIM242100) on chromosome 17p13 (Jobard *et al.*, 2002) and *CYP4F22* (MIM 604777) on chromosome 19p12–q12 (Fischer *et al.*, 2000; Lefèvre *et al.*, 2006). Furthermore, two additional loci have been described, the first on chromosome 19p13.2–p13.1 (MIM604781) (Virolainen *et al.*, 2000) which probably corresponds to the gene *CYP4F22* and the second on chromosome 12p11.2–q13 (Mizrachi-Koren *et al.*, 2005). In addition, mutations in *CGI-58/ABHD5* on chromosome 3p21 (MIM 275630) have been found to underlie Chanarin–Dorfman syndrome, a form of syndromic congenital ichthyosis with non-cutaneous characteristics involving liver, eyes, muscles, and nerves (Lefèvre *et al.*, 2001).

In 2002, we identified mutations in the genes *ALOX12B* and *ALOXE3*, in patients with non-bullous congenital ichthyosiform erythroderma from the Mediterranean area (Jobard *et al.*, 2002). These two genes encode the epidermal lipoxygenases 12R-lipoxygenase (12R-LOX) and lipoxygenase 3 (eLOX3). They are part of the LOX gene cluster of approximately 100 kb, which also contains the *ALOX15B* gene and the *ALOX15P* pseudogene. The *ALOX15B* gene, which encodes the 15S-lipoxygenase (15S-LOX-2), is located in the same orientation 25 kb downstream of *ALOX12B* and consists of 14 exons that are distributed over 9.7 kb of genomic sequence. Its involvement in ARCI has not been

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Abbreviations: ALOX, arachidonate lipoxygenase; ARCI, autosomal recessive congenital ichthyosis; SNP, single nucleotide polymorphism

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investigated so far. In this study, we report mutation analysis of the *ALOXE3*, *ALOX12B*, and *ALOX15B* genes in a total of 20 patients from 11 families.

RESULTS

All 20 patients were diagnosed as having ARCI based on common clinical criteria and had unaffected parents. The families were from Algeria, Italy, Morocco, Spain, Tunisia, and Turkey; nine of the 11 families were consanguineous. Most patients exhibited palmoplantar keratoderma with fissures, especially on the heel, and ichthyotic scaling of the scalp. The clinical details are presented in Table 1.

We excluded known loci on chromosomes 2, 3, 5, 12, 14, and 19 by genotyping and haplotype analysis in all the families reported here. Genotyping results in the consanguineous ARCI families I to VII showed homozygous haplotypes at the 17p13.1 locus defined by D17S1353, D17S1796, D17S1812, and D17S1805 for all patients except for family VIII in which a recombination was observed between D17S1812 and D17S1805 (Figure 1a). The genes *ALOX15B*, ALOX12B and *ALOXE3* are situated between the markers D17S1796 and D17S1812.

For the eight kindreds, mutation screening was performed in one affected child per family in which a homozygous haplotypes segregated. Sequencing of the 15 exons of *ALOXE3*, 15 exons of *ALOX12B*, and the exon–intron boundaries of the two genes revealed four variations in the coding sequence of the *ALOX12B* gene and two variations in the coding sequence of the *ALOXE3* gene (Table 2). The missense variations at codon 360, at codon 527, and a 2 bp deletion at codon 211, resulting in a premature stop codon at codon 239 in *ALOX12B*, appeared to be true mutations, as all affected children in the consanguineous families II, III, and IV were homozygous for those mutations and none of these novel sequence variations were found in 52 unrelated controls from North Africa. However, the variant P127S in *ALOX12B*, already reported by others as a mutation (Eckl *et al.*, 2005), and the variants I515V and R670W in *ALOXE3*, are likely to

Table 1. Clinical features

Family	I	II	IV	VI	VII	IX	X	XI
Mutation	No	ALOX12B	ALOX12B	No	No	ALOX12B	ALOX12B	ALOX12B
Collodion baby	No	No	No	No	Yes	Yes	Yes	Yes
Erythroderma	Yes	Yes	Yes	No	Yes	Yes	Mild	Mild
Type of ichtyosis now	NCIE	NCIE	NCIE	Lamellar	NCIE	NCIE	NCIE	Lamellar
Type of scales	Fine, pigmented, larger on legs and arms	Fine, white on thorax; large brown polygonal on the legs	Fine, white	Gray polygonal, xerosis of the skin	Fine, pigmented	Large brown	Mild scaling	Intense lamellar scaling, xerosis
Site	Whole body, more severe on legs and arms	Whole body, more severe on legs	Whole body, legs more severe on legs	Whole body including folds, face, scalp	Mainly arms and legs	Whole body including face	Whole body	Whole body, more severe on face and legs; xerosis on trunk and folds
Scalp	Extensive ichthyotic scaling	Extensive ichthyotic scaling	Extensive ichthyotic scaling	Ichthyotic scaling	Ichthyotic scaling	Mild	Mild scaling	No
Palmoplantar keratoderma	No	Yes, with fissures (heel)	Yes, with fissures (heel)	Hyperstriation of palmo-plantar, knees, fingers	Yes, with fissures (heel)	Yes, severe with fissures	Hyperlinearity and scaling	Hyperkeratosis and hyperlinearity
Remarks		Longitudinal striation of nails	Longitudinal striation of nails	Photosensibility		Convex nails, small stature	Light Photosensibility	
Histology	ND	Hyper and orthokeratosis; absence of stratum granulosum	Hyper and orthokeratosis; reduced stratum granulosum	Hyper and orthokeratosis, keratohyaline inclusions; abnormal stratum granulosum	Hyper and orthokeratosis; reduced stratum granulosum	ND	ND	ND

NCIE, non-bullous congenital ichthyosiform erythroderma; ND, no data.
No clinical data were available for families III, V, and VIII.

Table 2. Mutations and polymorphisms in the *ALOX12B* and *ALOXE3* genes in ARCI families

Family	Nb of affected	Origin	Consanguinity	Nucleotide change[1]	Site	Effect	Variation type[2]
I	1	Algeria	+	ALOX12B: c.[379 C>T] [=]+[=]	Exon 3	P127S	SNP (4%)
II	1	Algeria	+	ALOX12B: c.[632_633 delTC] [=]+[=]	Exon 5	F211CfsX29	Mutation
III	1	Morocco	+	ALOX12B: c.[1078 C>G] [=]+[=]	Exon 9	Q360E	Mutation
IV	2	Algeria	+	ALOX12B: c.[1579 G>A] [=]+[=]	Exon 12	V527M	Mutation
V	1	Algeria	+	ALOXE3: c.[1543 A>G]+[=]	Exon 11	I515V	SNP (1%)[3]
VI	4	Tunisia	+	ALOXE3: c.[2008 C>T] [=]+[=]	Exon 15	R670W	SNP (6%)
VII	1	Algeria	+	—	—	—	—
VIII	1	Spain	+	—	—	—	—
IX	2	Algeria	–	ALOX12B: c.[632_633 delTC]+[=]	Exon 5	F211CfsX29	Mutation
X right branch[4]	2	Italy	–	ALOX12B: c.[1261 C>T]+[1613 A>C]	Exon 9, exon 12	H421Y Q538P	Mutation Mutation
X left branch[4]	1	Italy	–	ALOX12B: c.[1261 C>T]+[2035 C>A]	Exon 9, exon 15	H421Y R679S	Mutation Mutation
XI	3	Turkey	+	ALOX12B: c.[1180 G>A] [=]+[=]	Exon 9	E394K	Mutation

ARCI, autosomal recessive congenital ichthyosis.
[1]According to the nomenclature of sequence variations, as provided by the Human Genome Variation Society (www.hgvs.org). (position 1=start codon). [=]+[=], the patient is homozygous for the variant; +[=], the patient is heterozygous for the variant.
[2]When the variation is a polymorphism, its frequency in the North African population is indicated in parentheses.
[3]rs3027205 in dbSNP database.
[4]See pedigree X in Figure 1b.

represent rare single nucleotide polymorphisms (SNPs) as they have been found at a frequency of 4, 1, and 6%, respectively, in controls from North Africa (Table 2). The SNP I515V in *ALOXE3* is reported in the dbSNP database as SNP rs3027205.

ALOXE3 and *ALOX12B* are part of the LOX gene cluster within a genomic segment of 100 kb at 17p13.1 (Krieg *et al.*, 2001). This cluster contains a third gene of the epidermis-type LOX family, *ALOX15B*, which is highly expressed in skin as well. The *ALOX15B* transcript (NM_00141) exhibits 92% identity with *ALOXE3* (NM_021628) and 78% identity with *ALOX12B* (NM_001139). Because no causative mutations in *ALOXE3* and *ALOX12B* were found in families I, V, VI, VII, and VIII, and because *ALOX15B* represents a strong candidate gene, we performed mutation screening in this gene in these five ARCI families. No mutations in the coding sequence and at the splice junctions of *ALOX15B* were found in the affected members of these families.

During the course of the study, three ARCI families from Algeria (family IX), Italy (family X), and Turkey (family XI) were identified (Figure 1b). Haplotypes analysis suggested linkage to chromosome 17p13 and excluded linkage to all other known loci on chromosomes 2, 3, 5, 12, 14, and 19. We undertook mutation screening in the LOX genes for those patients as well (Table 2). Of the two affected sisters in family IX, one died at birth and no material was available for genetic study. The second child was heterozygous for the 2 bp deletion in exon 5 of *ALOX12B* found previously in family II. Having sequenced all exons and the exon–intron boundaries of the gene, we could not find a second mutation in this patient.

Mutation analysis carried out in *ALOX12B* in family X from Italy revealed three novel compound mutations leading to amino-acid changes: the two affected sisters (right branch of family X, Figure 1b) had inherited the mutation H421Y from their father and the mutation Q538P from their mother. Their affected cousin (left branch of family X) carried the same mutation H421Y, inherited from her mother and a new mutation, R679S, inherited from her father. A mutation at codon 679 had been previously reported by others in an ARCI patient, but the sequence variation occurred in a different base of the codon, changing an arginine to leucine instead of serine (Eckl *et al.*, 2005). A sixth novel non-synonymous mutation was found in consanguineous family XI at codon 394 (E394K) (Table 2). None of these mutations were present in the 52 unrelated controls from North Africa.

DISCUSSION

By adding new variants to repertoire of *ALOX12B* mutations in non-bullous congenital ichthyosiform erythroderma, our data contribute to an enlargement of the spectrum of mutations for the development of efficient molecular genetic tests for analysis of at risk individuals whose carrier status is unknown. The clinical descriptions of patients (Table 2) did not permit a correlation between mutations in *ALOX12B* (Table 1) and a specific ARCI phenotype.

This is also the first study in which a possible role of *ALOX15B* in ARCI has been investigated. We were able to exclude the involvement of this candidate gene as well as of *ALOXE3* and *ALOX12B* in five ARCI families, indicating that the disease in these families must be caused by mutations in other genes inside the interval on chromosome 17p13. Haplotype analyses in the first eight families (I–VIII) provide strong evidence for the presence of a predisposing gene at

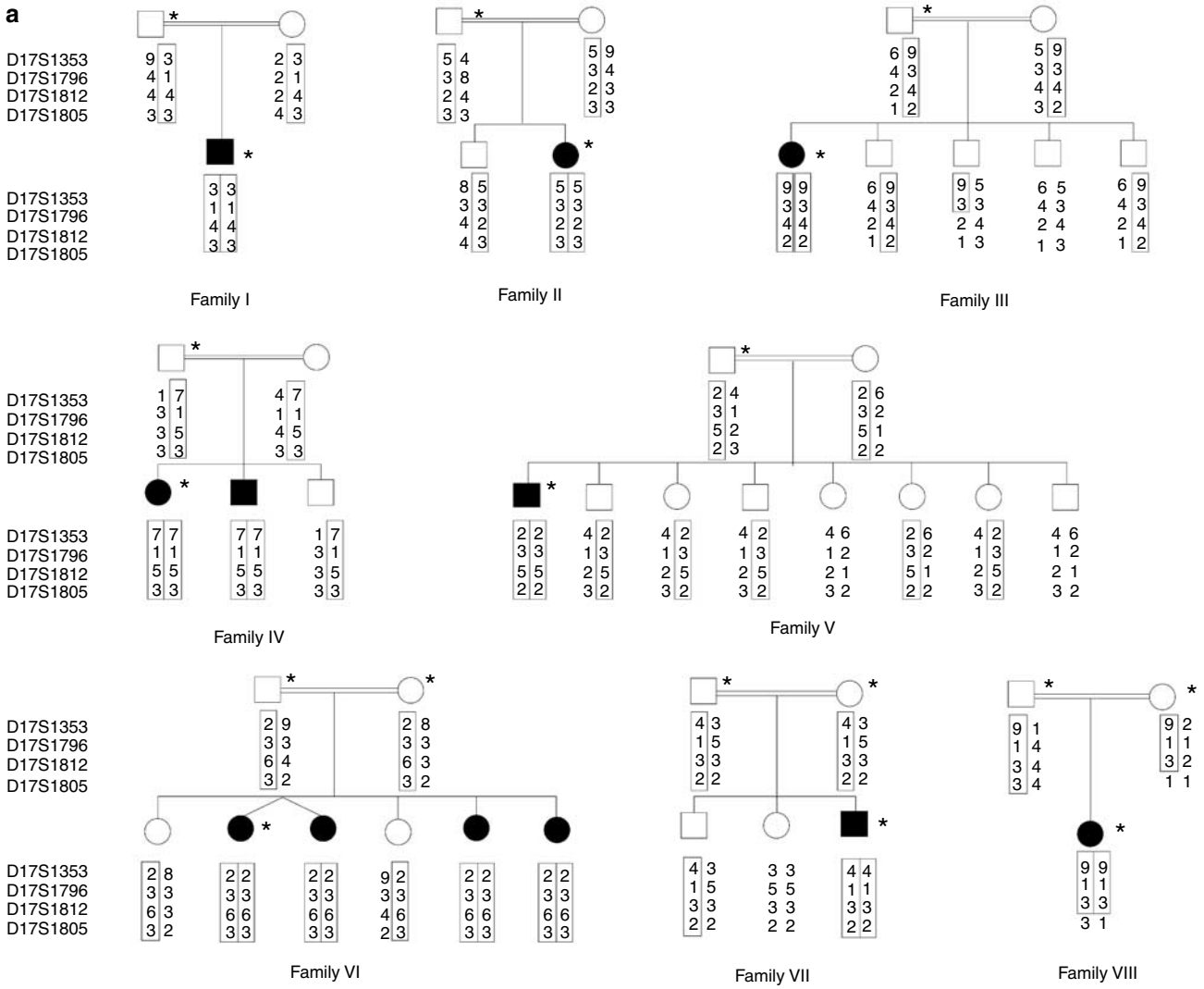

Figure 1. ARCI pedigrees. (**a**) Pedigrees and construction of haplotypes in the eight ARCI families used for haplotype analysis. Blackened symbols denote ARCI patients. Position of the markers from the p telomere of chromosome 17 from Golden Path (http://genome.ucsc.edu): D17S1353 at 7.55 Mb; D17S1796 at 7.73 Mb; D17S1805 at 8.52 Mb; and D17S1818 at 34.42 Mb. Affected haplotypes are boxed. (**b**) Pedigrees of the three recently identified ARCI families included in the mutation-screening panel. *Individual in which mutation analysis was performed. (Figure 1 continued on following page.)

this locus. However, we have found causative mutations in only three families, despite the informativeness of families V, VI, and VII. It is possible that the patients in the small consanguineous families with only one affected individual show homozygous haplotypes by chance, and the possibility of a linkage to a different locus cannot be formally excluded. Although unlikely, it is possible that patients of families I, V, VI, VII, VIII, and IX carry intronic mutations creating cryptic splice sites, or a mutation in the regulatory elements which might alter the expression of the proteins. One alternative hypothesis to explain possible linkage to this locus, as suggested by the haplotype analysis, would be the presence of another gene involved in predisposition to the disease in the same interval. To date, seven known genes have been characterized between D17S1353 and D17S1796, 20 genes between D17S1796 and D17S1812, and five genes between D17S1812 and D17S1805.

The affected child in consanguineous family I was homozygous for the variant P127S in *ALOX12B*, and the affected child in family VI was homozygous for the variant R670W in *ALOXE3*, and in both cases the unaffected parents were found to be heterozygous for the variant. We believe that it is quite unlikely that these sequence variations constitute the causative mutations as they were found to have a frequency of 4 and 6%, respectively, in the North African control population. However a recent report described a self-healing collodion baby of Turkish origin, who was heterozygous for the variant P127S in *ALOX12B* and for which *a* second mutation was not found (Eckl *et al.*, 2005). The authors postulated that this variant was responsible for the instability of the protein in the mutant cells and the lack of expression of the protein. Our findings would suggest that, if confirmed, this dysfunction of ALOX12B would be due to another unknown mutation rather than to the SNP P127S itself.

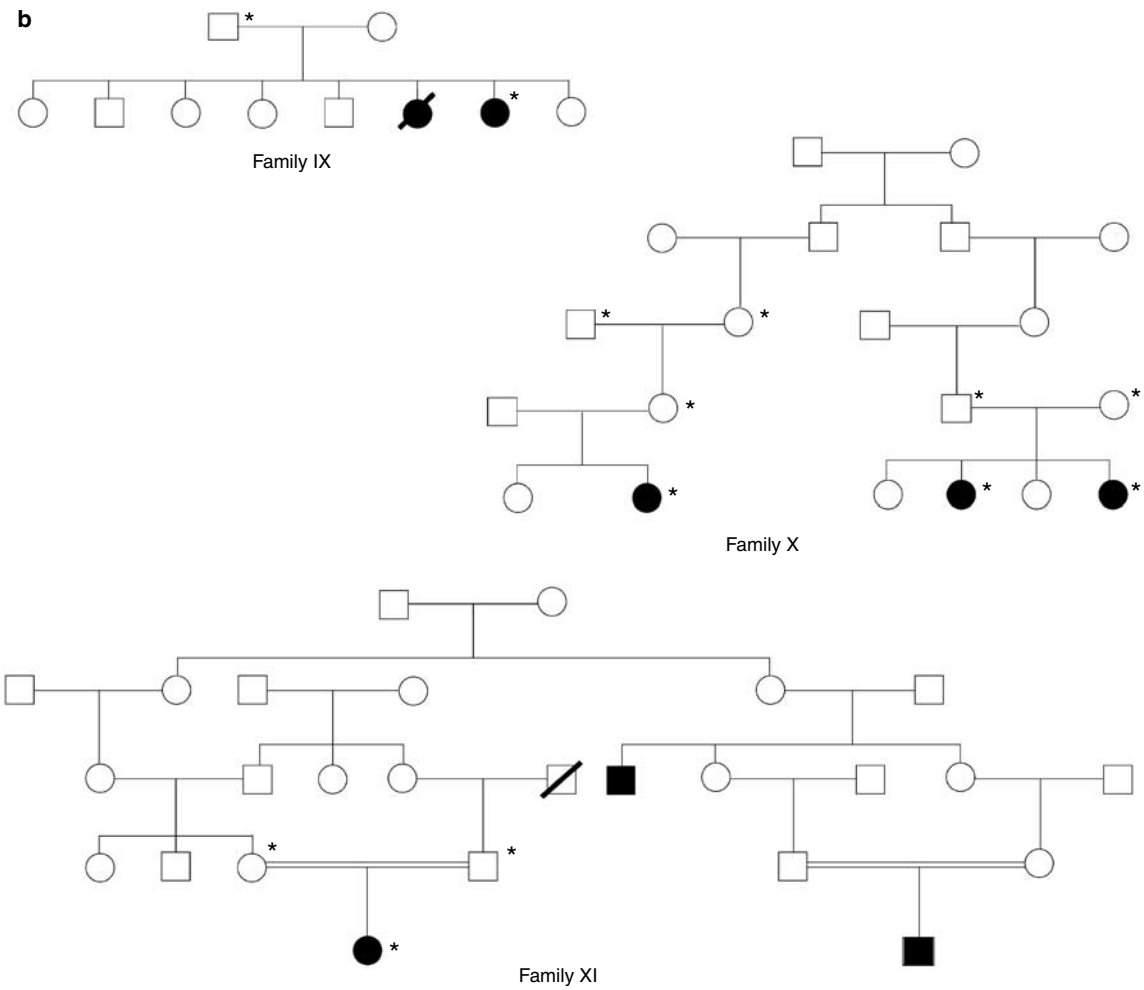

Figure 1. Continued.

Identification of seven novel mutations in *ALOX12B* in families from Algeria, Italy, Morocco, and Turkey brings the total number of reported mutations in this gene to 20; seven point mutations have been reported in *ALOXE3* (Jobard *et al.*, 2002; Eckl *et al.*, 2005). The patient in family IX was heterozygous for the c632_633 delTC mutation and we did not find a second mutation in another exon of the gene. It is unlikely that this variation corresponds to a rare polymorphism as it has been found in the homozygous state in family II, and was not present in the 52 controls (104 chromosomes) from North Africa that were sequenced. A more plausible explanation would be the presence of a second undetected mutation within the intronic sequence creating a cryptic splice site, or within the regulatory sequences leading to a lack of expression of the protein. The possibility of a large deletion, which would be undetectable when sequencing the exonic regions separately, cannot not be discounted either.

Recently, Yu *et al.* (2005) have shown that the previously reported mutations in the *ALOXE3* and *ALOX12B* genes completely eliminate the enzymatic activities of the two epidermal lipoxygenases. Although we have not analyzed the biochemical effect of the novel *ALOX12B* mutations presented here, we can postulate that they also have deleterious consequences on enzyme function: the deletion c632_633 delTC leads to a premature stop codon upstream from the lipoxygenase activity domain. The six missense mutations Q360E, E394K, H421Y, V527M, Q538P, and R679S are all located within the lipoxygenase catalytic domain, and amino-acid changes may alter the folding of the protein, and therefore abolish or alter ALOX12B activity (Figure 2). Taken together, these genetic and biochemical insights provide evidence for the involvement of lipoxygenases in the formation of the skin permeability barrier.

MATERIALS AND METHODS

Genetic analysis

DNA was extracted from whole blood using standard procedures after informed consent of the patients and/or their parents. The medical ethical committee of AFM/Généthon approved the described study. The study was conducted in concordance with the Declaration of Helsinki Principles. Genotyping using an exclusion panel of 36 fluorescent microsatellite markers at known loci on chromosomes 2, 3, 5, 12, 14, 17, and 19 was carried out in families I–VIII as described previously (Fischer *et al.*, 2000). The set of

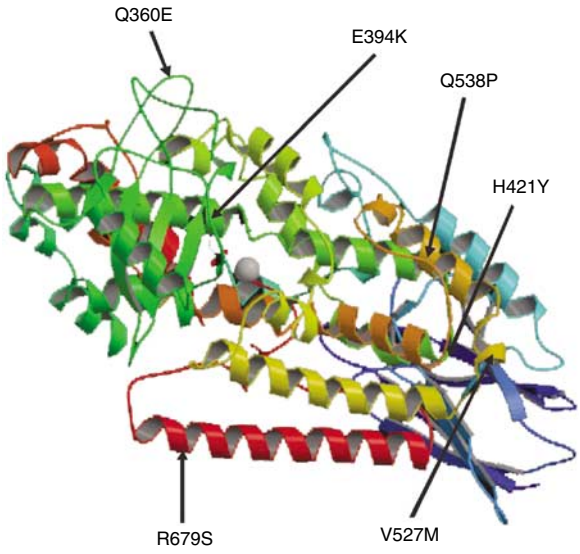

Figure 2. Location of novel point mutations within the structural model of LOX proteins. Positions equivalent to the mutated residues are shown on the crystal structure of rabbit reticulocyte 15-LOX (according to the RCSB Protein Data Bank, http://www.pdb.org/pdb/cgi/explore.cgi?pdbId=1lox). Gray: iron. Mutations E394K, V527M, Q538P, and R679S are located in helix fragments, whereas mutations Q360E and H421Y are located in turn fragment. All mutations are located within the lipoxygenase catalytic domain.

microsatellites used includes markers D17S1353, D17S1796, D17S1812, and D17S1805 around the epidermis-type LOX gene cluster. In addition to this panel, a genome-wide scan was performed on families IX, X, and XI using 430 highly polymorphic microsatellite markers from the ABI panel (Linkage Mapping Set2, LMS2, Applied Biosystems, Foster City, CA). Haplotypes were constructed assuming the most parsimonious linkage phase.

Mutation screening

We sequenced at least one affected individual per family and one or both parents. Oligonucleotide primers for *ALOX12B* and *ALOXE3* genes flanking the coding exons and internal primers for sequencing were those described previously (Jobard *et al.*, 2002). Primers and PCR conditions used for the screening of the *ALOX15B* gene are given in the Table S1 available online. Both strands from all subjects and controls were sequenced for the entire coding region and the intron–exon boundaries.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

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ELECTRONIC DATABASES

GenBank accession numbers: *ALOXE3*, NM_021628.1; *ALOX12B*, NM_001139.1; *ALOX15B*, NM_001141.1
OMIM, http://www3.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
dbSNP, http://www.ncbi.nih.gov/SNP

SUPPLEMENTARY MATERIAL

Table S1. Primer sequences for the *ALOX15B* gene.

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